

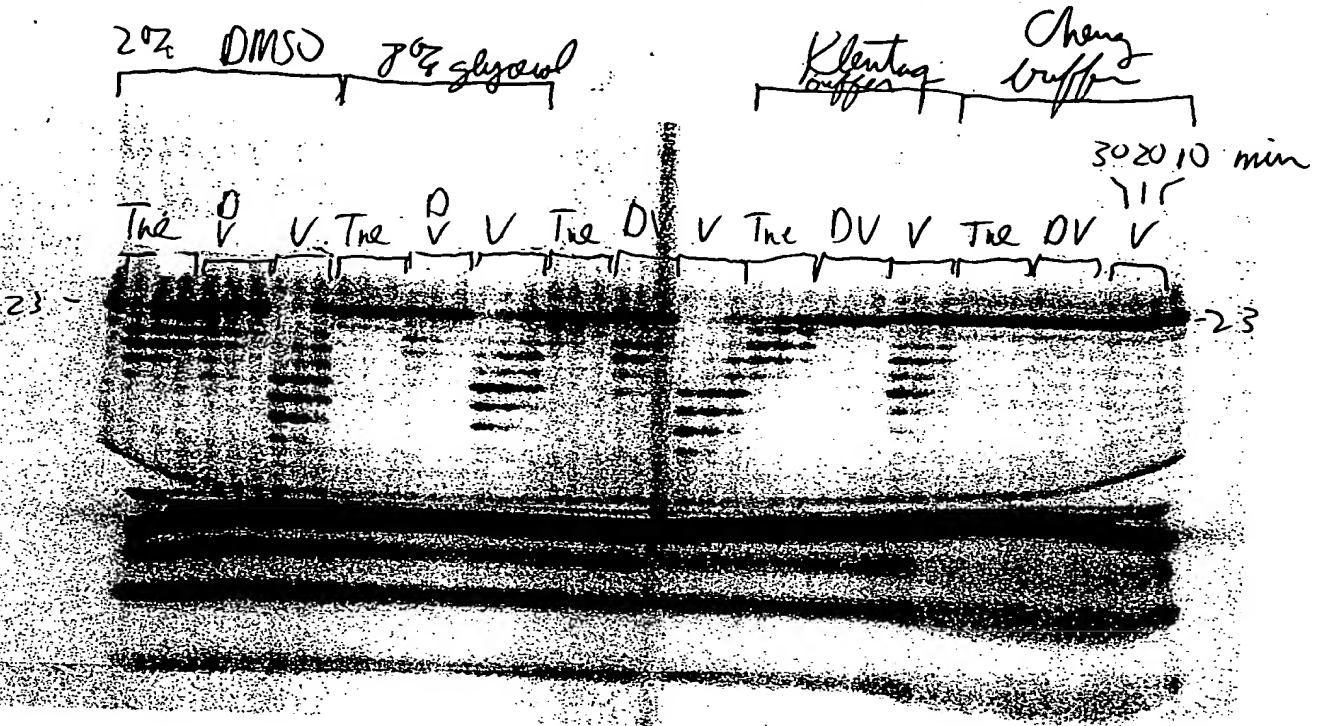
82

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



Result.

T Pag N .

Witnessed & Understood by me,

Deena Polarp

Date

11/29/94

Invented by

Recorded by

Date

11/5-94

From Page No.____

	Chem	Klentz	Vent
Inhib pH 8.7	20 mM		
Trio-Hill pH 9.1		50 mM	
K OH ^{pH} _{P.H}	85		20 mM
K OH ^{pH} _{P.H}			10
(NH ₄) ₂ SO ₄	1.2	16	10
Mg(OAc) ₂		1.2	2
MgSO ₄	2 %		0.1 %
DMSO			
Inhib			
Tween 20/MPD		0.1 % from top	
glycerol	8 %	1	1
same strength	105	82	50
Vent	—	+	++
Deep vent	—	—	+
Tue	—	+	—

(+ for DMSO)

T	Pag	Nº
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
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92	92	92
93	93	93
94	94	94
95	95	95
96	96	96
97	97	97
98	98	98
99	99	99
100	100	100

Witnessed & Understood by me,

Deirdra Polay

Date

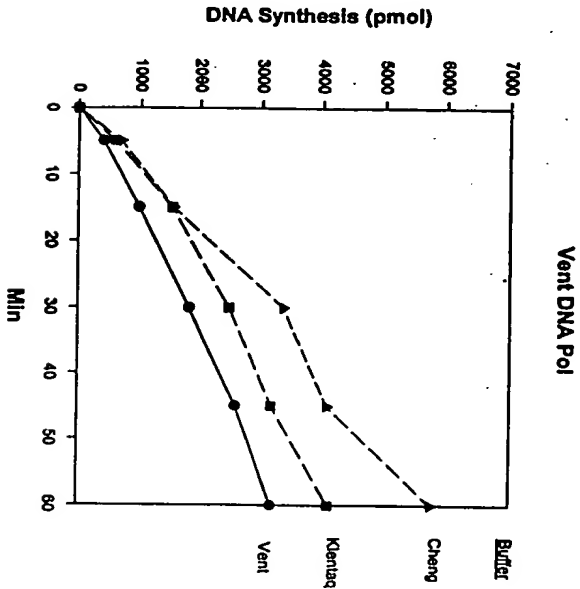
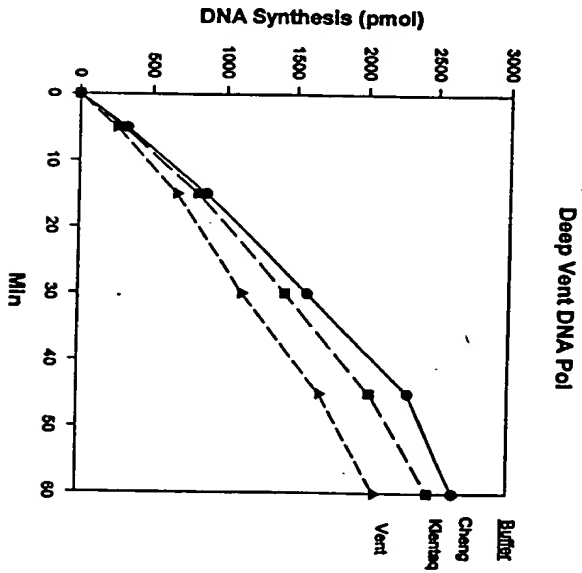
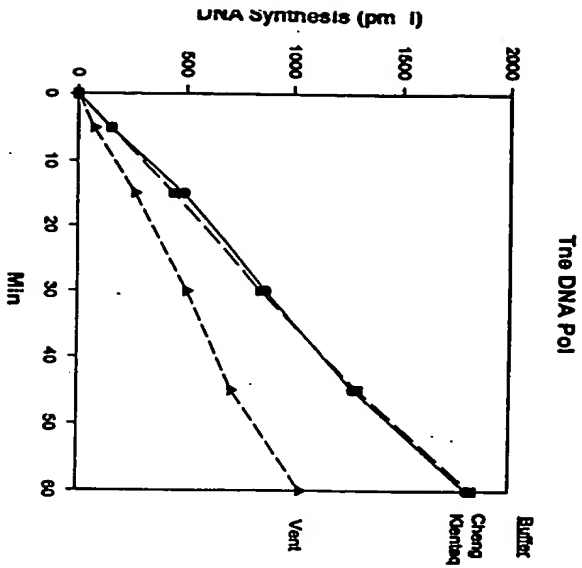
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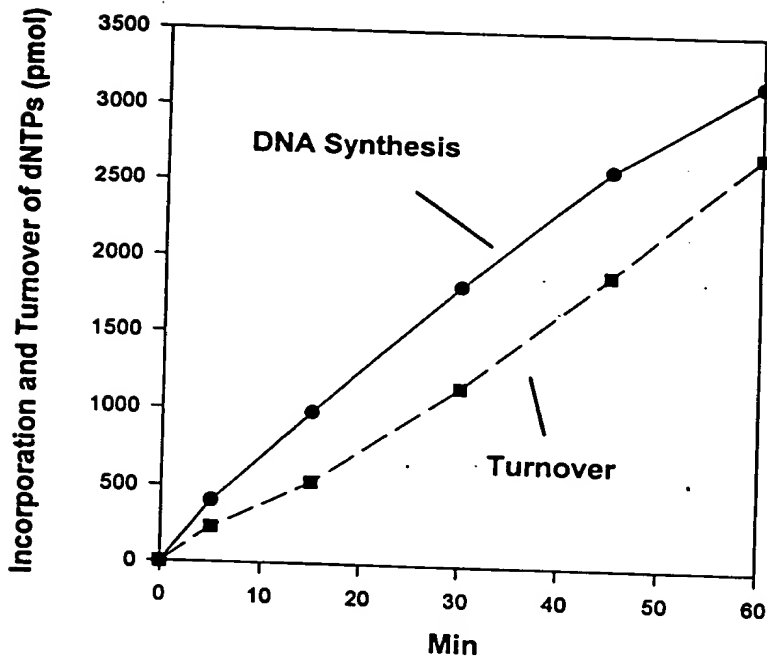
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In each case, DNA synthesis is lower in
 Primer degradation was highest in Vent

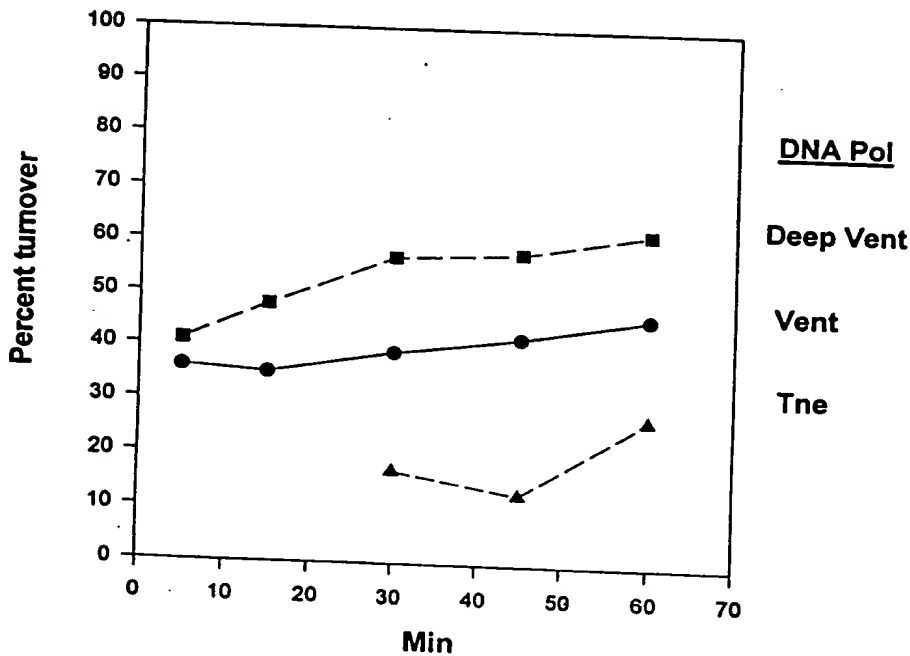
got Turnover
 very DNA synthesis
 & label

Vent DNA Pol in Vent Buffer



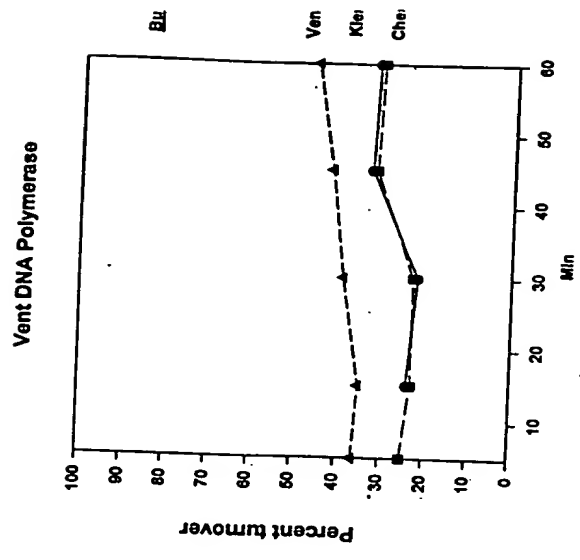
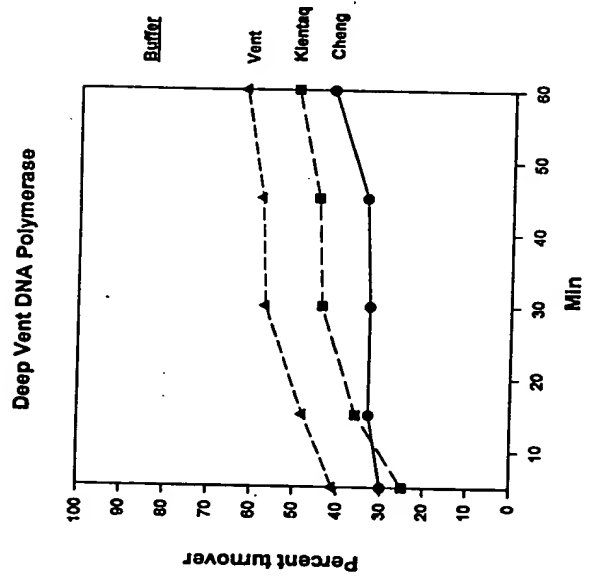
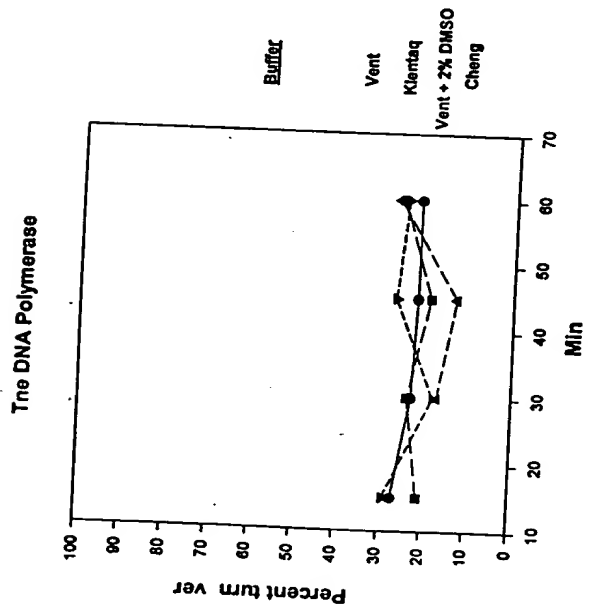
DNA synthesis
and turnover
to dNMP

Activity in Vent Buffer



$$\text{Percent turnover} = \frac{\text{turnover}}{\text{incorporation} + \text{turnover}}$$

Deep Vent has
higher turnover
than Vent as
expected. Tne
is ~2x lower
than Vent and
Deep Vent



effect of buffer on turnover is not large compared to effect on primer degradation

ss d & Understood by me,
Charles Polcup

Date
 11/29/94

Invented by
[Signature]
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Date
 11-5-94

Turnover for Vent, deep Vent
(follow p. 61, 7)

From Page No. _____

	(A)	(B)	(C)
H ₂ O	359	487	489
5x Cheung buffer	133	467	47
10x Klenow		66.7	
10x Vent buffer			66.7
Tag storage buffer	6.71		
5.7 mg/ml actin	90		
DNA			
4-ATG-T-TP 10mM each	3.33		
32P dATP 10mCi/ml	1.21		
Mg(OAc) ₂ 50 mM	16 μ l		
MgSO ₄ 100mM		8 μ l	
DM50 10.0%			
	0.65 ml	0.633	2.633.65 use 1.
	(1) (2) (3)	(4) (5) (6)	(7) (8)
Tag storage buffer	195 195 195	190 190 190	190 190
Vent 0.08 μ l	4	4	4
Deep Vent 0.08 μ l	4	4	4
Taq 0.07 μ l	4	4	4
H ₂ O	4	4	4
min to 70°C, start by addition of pol			
remove 15 μ l to 5 μ l 0.2 MEDTA \rightarrow spot 15 μ l on G			
and remove 5 μ l to 5 μ l Kill solution (20 μ mol/ml DA			
100 mM EDTA) at:			
0 5 15 30 45 60 min			
spot 2 μ l on PEI			
resolve in 1m LiCl			
* dilutions of pol			
name as P.81			

Results: see graph on P.81

Witnessed & Understood by m ,

Deena a Polay

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T Pag

ag N

(D)

14.4

✓

✓

✓

66.7 20

✓

→ 27

✓

1 μ l / 100 μ l PCR \Rightarrow Cf = 0.005% Tween 20 / NP40
 this makes up for no TPE here - its present in Joes long PCR Run.

→ 1

✓

(Cp = 50 μ m each)

→ 0.36

✓

(220 x 10⁶ total cpm)

✓

(1.2 mM Mg(OAc)₂)

✓

(1.2 mM MgSO₄ in Klenow buffer)4 μ l

✓ Cf =
(2% DMSO)

(2 mM MgSO₄ in 1X Vent buffer)

(10)

19.4

✓

(0.4 units total of each pol)

4

1

To Page No.

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Date

11/29/94

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11-9-94

86

min

Project No.

060

Book No.

Turnover TITLE

Incorp

Ampl

From	To	Amount	Book No.	Turnover	Title	From	To	Amount	Book No.	Turnover	Title
01	01	543.00	BK60	(14)		61	61	269.00			
02	02	650.00	110	24		62	62	7412.00			
03	03	1014.00	476	22		63	63	16953.00			
04	04	1485.00	771	34		64	64	36825.00			
05	05	2627.00	2148	32		65	65	44610.00			
06	06	3187.00	2725			66	66	62771.00			
07	07	525.00	BK60			67	67	241.00			
08	08	662.00	141	30		68	68	3518.00			
09	09	948.00	436	33		69	69	9506.00			
10	10	1271.00	763	33		70	70	17320.00			
11	11	1677.00	1188	34		71	71	25050.00			
12	12	2340.00	1871	42		72	72	28643.00			
13	13	624.00	BK60			73	73	324.00			
14	14	694.00	72	(32)		74	74	1974.00			
15	15	796.00	177	27		75	75	5340.00			
16	16	880.00	264	23		76	76	9478.00			
17	17	976.00	363	22		77	77	13880.00			
18	18	1110.00	501	22		78	78	19753.00			
19	19	805.00	BK60	775 Ave		79	79	321.00			
20	20	977.00	192	25		80	80	8826.00			
21	21	1409.00	467	23		81	81	23029.00			
22	22	1803.00	762	23		82	82	37324.00			
23	23	2832.00	1533	32		83	83	47661.00			
24	24	3299.00	1873	31		84	84	61758.00			
25	25	774.00	BK60			85	85	404.00			
26	26	918.00	99	25		86	86	4493.00			
27	27	1406.00	415	36		87	87	12238.00			
28	28	2277.00	1118	44		88	88	21497.00			
29	29	2989.00	1551	45		89	89	30491.00			
30	30	4085.00	2472	50		90	90	36800.00			
31	31	777.00	BK60			91	91	214.00			
32	32	813.00	21	(12)		92	92	2257.00			
33	33	947.00	121	21		93	93	6671.00			
34	34	1136.00	263	24		94	94	12685.00			
35	35	1204.00	314	19		95	95	19429.00			
36	36	1631.00	633	26		96	96	27534.00			
37	37	919.00	BK60	922 Ave		97	97	239.00			
38	38	1284.00	231	36		98	98	7128.00			
39	39	1754.00	530	35		99	99	17335.00			
40	40	2728.00	1150	39		100	100	32171.00			
41	41	3910.00	1903	42		101	101	45795.00			
42	42	5168.00	2704	46		102	102	56065.00			
43	43	924.00	BK60			103	103	318.00			
44	44	1205.00	180	41		104	104	4474.00			
45	45	1892.00	617	48		105	105	11839.00			
46	46	3234.00	1472	57		106	106	19756.00			
47	47	4572.00	2325	58		107	107	29674.00			
48	48	6365.00	3467	62		108	108	36540.00			
49	49	863.00	BK60			109	109	261.00			
50	50	901.00				110	110	1566.00			
51	51	953.00	20	(7)		111	111	4647.00			
52	52	1083.00	103	17		112	112	8879.00			
53	53	1085.00	103	13		113	113	12496.00			
54	54	1529.00	386	27		114	114	18327.00			
55	55	984.00	BK60			115	115	295.00			
56	56	891.00				116	116	1709.00			
57	57	1067.00	92	29		117	117	4261.00			
58	58	1086.00	104	18		118	118	8343.00			
59	59	1336.00	264	37		119	119	12504.00			
60	60	1467.00	347	25		120	120	18443.00			

With

Danaa Pump

Date

11/29/94

Invented by

Recorded by

11/9-94

Fig N. _____

JAMP BK60%

1. Chemo mix = 564 ave
2. Blank mix = 785
3. Vent mix = 922

Spot
Chemo

Blank

Vent

$$7582 \text{ cpm} \left(\frac{50 \mu\text{L Rxn vol}}{2 \times \text{spotted}} \right) \left(\frac{200}{195} \right) \left(\frac{1}{2500 \text{ pmol}} \right) \left(\frac{1}{4} \right) = 194 \frac{\text{cpm}}{\text{pmol at pmol}}$$

$$267 \frac{\text{cpm}}{\text{pmol}}$$

$$314 \frac{\text{cpm}}{\text{pmol}}$$

pmol incorp =
(200 μL Rxn)

$$\frac{\text{cpm}}{\text{cpm/pmol}} \left(\frac{200}{15} \right) \left(\frac{20}{15} \right)$$

pmol turnover =
20 μL Rxn

$$\frac{\text{cpm} - \text{BK60}}{\text{cpm/pmol}} \left(\frac{200}{5} \right) \left(\frac{10}{2} \right)$$

$$\% \text{ turnover} = \frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$$

121 75821.00
122 104512.00

T Page No. _____

Read & Understood by me,

Date

Invented by

Date

re: Polap

11/29/94

Rec rd d by

11-10-94

PAGES 88-89 OF NOTEBOOK WERE BLANK

ATGAM, carried Project No. _____
90 out at Frederick. Book No. _____

TITLE Repeat unit assay QC for rTag
lot # EKBT1 done on P 61 1/2/94

Form Page No. _____

Amplitag lot # 9957 for control

lot EKBT1 is ~ 4.01 u/ml based on P. 61

1. starting dilutions of EKBT1:

1:80 (estimate cf = 5%)

1:160 (estimate cf = 2.5%)

lot EKBT1 5 µl

Tag storage buffer 395 µl

Vf = 400 µl

actual is 4.03 u/ml

5 µl

795 µl

Vf = 800 µl

actual is 2.01 u/ml

2. 1/600 dilutions

serial dilution #	1-6	7-12	13-17	19-24	25-30	31-36	37-42	43-48	49-54
	I	II	III	IV	V	VI	A-1	A-2	A-3
1:80 dil	3A	3	3						
1:160 dil				3	3	3			
Amplitag 5% lot #							3	3	3

Dilution buffer 1797 µl
Vf = 2000 µl
1800 µl

Vortex 5A
use from 16
20 and 40 ml

dilute I - A-3 as shown for I below:

3. Serial dilutions

serial dilutions #	dilution buffer
1	100 µl
2	100 µl
3	100 µl
4	100 µl
5	100 µl
6	1 ml of I

dilute I - III and assay
then dilute IV - VI and assay
then dilute A-1 - A-3 and assay

SA I-III = 45 µl assay mix + 5 µl dil buffer, do same for IV-VI
spot 4x 5 µl on 6 EC in one aqueous

Blank is 45 µl assay mix + 5 µl dil buffer → spot on 6 EC along with other

Witnessed & Understood by me,

Deborah Brown

Date

1/6/95

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R c rd d by

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11-15-94

T Pag N

age N _____

55-57 = Blank for I-III, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amphotag lot #9957 here gives a
unit value of ~~323.4 u/ml~~ 323.4 u/ml
compared to 401 u/ml (found on P.61, 10-1-94)

To Page No. _____

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Date

Inv nted by

Date

Recorded by

10-15-94

Sandra Pokany

11/6/95

Project No. _____

Book No. _____

TITLE

New definition of EKBT1 to
for Larry Mestey

From Page No. _____

* will use old unit value of 401 u/l no can use
old definition of Jerry used in October
(see P 91 where final unit determination for EKBT1
is 323.4 units/ μ l)

Tag storage buffer

391 μ lTag lot # EKBT1
("401" u/ μ l)10 μ l

* see above

$$VF = 401 \mu\text{l} \quad \left(\frac{10 \text{ units}}{\mu\text{l}} \right)$$

Calibrated P20 (P20
its exactly 10 μ g for
for P1000 (P2077) use
378 μ l which gives
391 μ g

1. Bring Tag storage buffer to room Temp.
2. Bring small aliquot aliquot of EKBT1 (main stock)
to room Temp.
3. deliver 10 μ l Tag into 391 μ l storage buffer, rinse
~10 times (see Triturate)
4. mix with P1000 to get in all storage buffer
5. vortex 5 sec
6. mix end over end in cold room 2 hr

With ssed & Und rsto d by me,

Deeana Polansky

Dat

1/6/95

Invented by

R. J. P.

R c rded by

T Pag No

Dat

11-30-94